PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference CU2003/254/PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/CU2004/000012	International filing date *(day/month/year)* 03.11.2004	Priority date *(day/month/year)* 04.11.2003

International Patent Classification (IPC) or national classification and IPC
INV. C07K14/22 A61K39/00

Applicant
CENTRO DE INGENIERIA GENETICA Y BIOTECNOLOGIA

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in celectronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☒	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.04.2005	30.03.2006

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CU2004/000012

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-20	as originally filed
Claims, Numbers	
1-12	received on 20.04.2005 with letter of 18.04.2005
Drawings, Figures	
1-11	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing .

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	2-4, 6-12
	No:	Claims	1, 5
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

1 The following **documents** are referred to;

D1: EP-A-1 297 844 (Microbiological Research Authority)
D2: Biochemical and Biophysical Research Communications, G. Sardiñas et al. vol. 277, pages 51-54 (2000)
D3: US-A-2003/0059444 (Zollinger et al.)
D4: Infection and Immunity, Saunders et al. Jan. 1999, pg. 113-119, vol. 67. no. 1
D6: WO-A-03 051 379 (Microbiological Research Authority)
D7: WO-A-01 91 788(Statens Insititutt for Folkehelse)
D8: WO-A-01 09 350 (Smithkline Beechan Biologicals S.A.)
D9: Infection and Immunity, Jin et al., vol. 71, no. 9, pages 5115-5120, Sept. 2003.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2 **NOVELTY** (Art. 33(2) PCT)

2.1 D1 discloses compositions comprising *N.meningitidis* outer membrane vesicles (OMV) enriched with antigenic components used in the form of a vaccine in order to elicit an immune response. The antigens used in the present case are the Transferrin binding proteins TbpA and TbpB. The enrichment is achieved by mixing the OMV with the antigens.

2.2 In view of the prior art cited, claims 1 to 12 appear to be novel and meet therefore the requirements of Art. 33(2) PCT, since the prior art does not disclose vaccines in which the antigen has been incorporated into the bacterial outer membrane vesicle by co-folding such that the vesicle structure is maintained intact.

3 **INVENTIVE STEP** (Art. 33(3) PCT)

3.1 Documents D3 and D4 are considered to represent the most relevant state of the

art and disclose a vaccine using native outer membrane vesicles (NOMV) for Neisseria or other Gram negative bacteria. D3, Page 1, paragraph 8, states that "the antigens presented as part of the NOMV... are in a completely native configuration and environment as part of intact outer membrane".

3.2 The difference between the subject-matter of the present application and that disclosed in the prior art is that the prior art methods involve the generation of OMVs from genetically modified bacteria, compared to the present application which involves the incorporation of the antigens into the OMV.

3.3 The problem to be solved by the present invention may therefore be regarded as providing a method of incorporation of protein antigens into outer membrane vesicles.

3.4 The proposed solution is the incorporation of an antigen into a bacterial outer membrane vesicle using the method of claim 1 such that the vesicle structure is maintained intact and proper folding of the antigen is achieved.

3.5 D2 describes the conjugation of the P64k peptide from *N.meningitidis* strain CU385 (B:4:P1.19, 15) outer membrane vesicles (OMV; as used in the present application) used as a carrier to two cyclic synthetic peptides derived from variable regions of the outer membrane protein PorA. The P64k was found to be an efficient carrier protein for PorA derived peptides. The chemical conjugation to the carrier did not affect the folding and allowed the synthetic peptides to induce a PorA-specific immune response.

3.5.1 Although the person skilled in the art is aware of the use of OMV as an effective carrier for antigens, resulting in an effective induction of an immune response, D2, the antigen was chemically conjugated to the OMV for use as a carrier in the prior art disclosure compared to the present application where the antigen is incorporated into the OMV by co-folding.

3.5.2 The use of outer membrane vesicles from gram negative bacteria is commonly

known in the state of the art for use as vaccines, see D6 to D8, to name but a few, however in the present application the OMV are not used as carriers but are used to refold the antigens which are incorporated into the OMV structure.

3.5.3 Furthermore, when considering the state of the art, the person skilled in the art could not have anticipated that, as demonstrated in the application Example 4, that after incorporation of the TbpB in the OMV of a heterologous meningococcal strain, all variants used for the immunization were able to induce blocking antibodies that were able to inhibit the binding of human transferrin to the meningococcal transferrin receptor, indicating the functional activity of the antibodies. In fact the mixture of Tbps with OMV prepared according to the method of claim 1 was found to confer higher protection than the antigen Tbps alone.

3.5.4 However the above effect is only demonstrated with the insertion of the TbpB protein into OMV of *N. meningitidis*, Example 4 and Example 6 involving the incorporation of PorA into OMVs from *Neisseria lactamica* and *Branhamella catarrhalis*. Consequently the subject-matter of claims 2 to 4 and 6 to 12 are considered to involve an inventive step as required by Article 33(3) PCT.

3.5.5 The IPEA considers the extrapolation of the method and vaccine of the present application to encompass the incorporation of **any** antigen into **any** bacterial outer membrane vesicle as being purely speculative and not based upon any technical evidence or facts.

3.5.6 Consequently the subject-matter of claims 1 and 5 have not been demonstrated as solving the above defined problem and therefore cannot be recognized as involving an inventive step according to Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

CLARITY (Art.6 PCT)

4 Claims 1 and 5 encompass **any** antigen incorporated into **any** Gram-negative bacterial OMV, whereas the description and examples actually only involve the incorporation of PorA into OMV from *Neisseria meningitidis*, TbpB and PorA into OMV from *Neisseria meningitidis* and a synthetic peptide containing the variable region 2 derived from the surface loop 4 of class 1 OMP inserted into OMV from *Neisseria meningitidis*.

4.1 Therefore all the exemplified antigens are derived from *Neisseria meningitidis* and inserted into the OMV from *Neisseria meningitidis*. There is no technical evidence to indicate that the method would succeed if carried out using any other antigens or any other gram negative bacterial OMV. The general discussion in the description page 7, lines 18 to 21 is not considered sufficient to infer that simply if the method is effective with one member of Gram negative bacteria, that one may assume that the method may be successfully used with any antigen and any Gram negative bacteria.

4.2 The subject-matter of claims 1 and 5 therefore appears to be entirely speculative, not based upon technical facts and not supported by the description according to Article 6 PCT.